Overview

Alabama experienced *Karenia brevis* red tide events in 1995-1996, 2000, 2005, 2007-2008 and 2015-2016. Shellfish harvest areas were closed. Tourist and resident populations were affected by the aerosols which caused respiratory irritation. Several public health advisories were issued by the state health officer as cell counts rose in shellfish areas and on Baldwin County beaches. Fish kills caused by the dinoflagellates, *Alexandrium monilatum and Karlodinium veneficum,* and the flagellate, *Chattonella subsalsa* have been documented at Gulf Shores, Weeks Bay, and the Theodore Industrial Canal. *Prorocentrum concavum* bloomed concurrently with the *Karenia* bloom of 2007 in Bon Secour Bay and required toxin testing by the FDA. The saxitoxin producer, *Pyrodinium bahamense*, was identified during routine shellfish growing area monitoring in 2007 and was found to be toxic at the lower levels of detection.

The diatom, *Pseudonitzschia*, has been identified in blooms along the Gulf in the Little Lagoon area. Events in 2005 and 2013 showed low levels of the toxin, domoic acid. It is important to be aware of this phenomenon as domoic acid is the agent of amnesic shellfish poisoning.

In 2006 there was a report of sewage at the site of a moored barge in the Intercoastal Canal in Baldwin County, Alabama. Black, stinking, floating material was reported by a citizen and investigated by health department environmentalists. *Lyngbya*, a cyanobacterium, was identified. Some cyanobacteria produce toxins capable of promoting tumors and causing severe damage to internal organs. They may grow as mats that appear to be floating sewage or fecal material in marine and estuarine waters. Even in the absence of mat production the cyanobacteria may produce toxins causing symptoms such as contact dermatitis, respiratory, eye and other mucous membrane irritation, and if water is ingested, gastrointestinal problems. Dried mats of cyanobacteria on the beach can cause toxic responses if handled. The cyanobacteria are included in the Alabama HAB surveillance because of these potential public health impacts.

The recounting of these events and the toxin details is not meant to alarm, but to promote awareness and preparedness for events. The *Karenia brevis* blooms required state, federal, and local agencies to implement plans to reduce impact to public health, provide information about the blooms and their duration, and assess long term effects. This document will attempt to codify these procedures for reporting a HAB event, collection and analyses of samples, public health response, and pathways of communication.

Goals

The primary goals of the Alabama HAB Response Plan are:

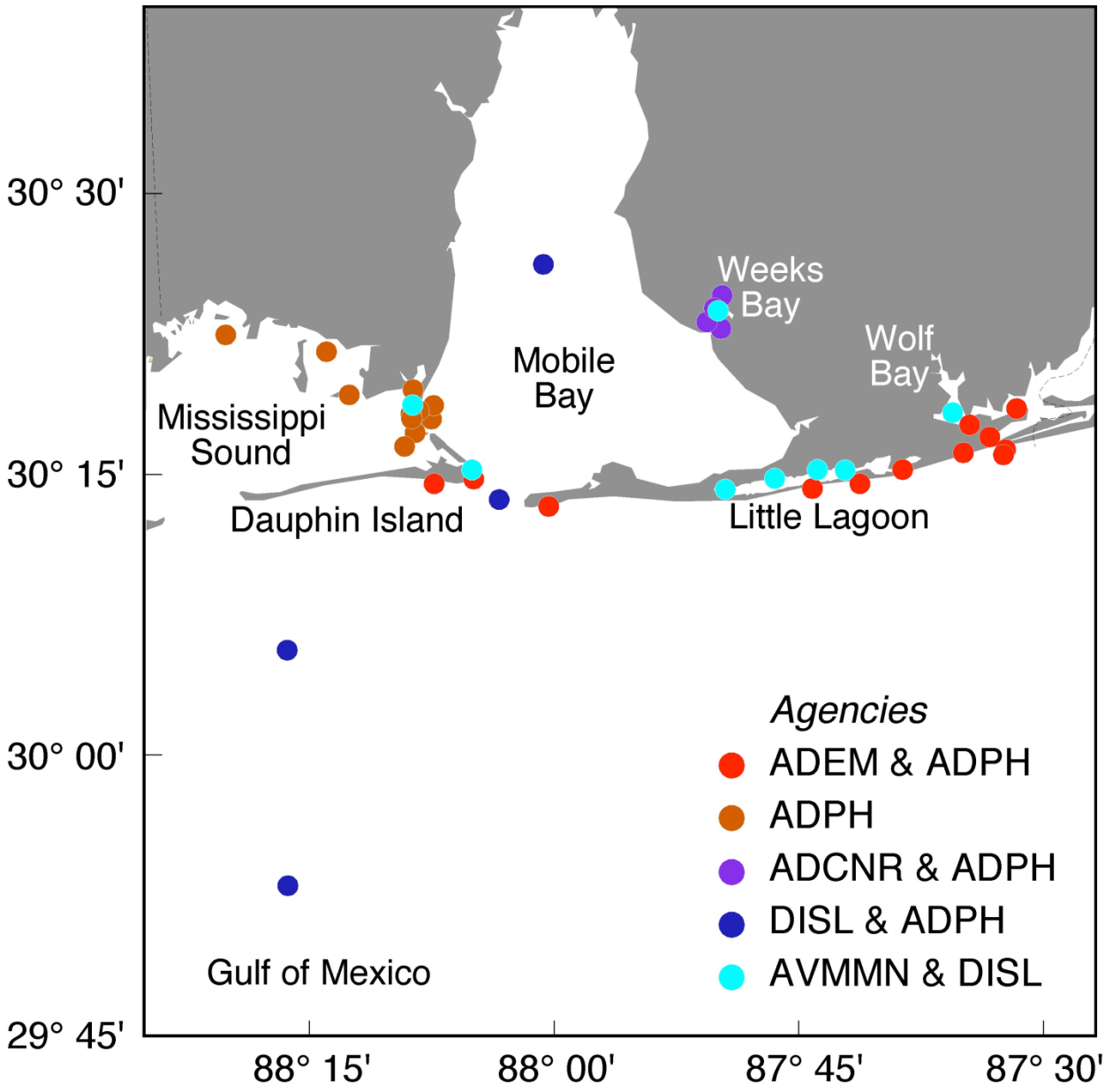
1. Provide accurate information regarding HAB to local, state, federal and academic agencies in Alabama
2. Provide timely health advisories associated with HAB and human health.
3. Assure regulation of the shellfish harvest as required by the Marine Biotoxin Control Plan within the National Shellfish Sanitation Program.
4. Define contacts and roles within county, state and federal agencies that respond to HAB events
5. Provide, in a timely manner, accurate analyses of toxins and organisms using standardized methodologies for identification and quantification
6. Maintain databases of HAB monitoring and event response data in Alabama coastal waters.

Management of HAB in Alabama is an integrated effort conducted by a number of state, federal and academic agencies.

Agencies

- Alabama Department of Public Health
    - Seafood Branch- This branch of Environmental Health has responsibility for the shellfish growing areas and issues public health advisories in the event of a *Karenia brevis* red tide. Samples are routinely collected from specific shellfish areas to reflect the potential HAB in harvest waters. Samples are collected 5 times per year from sentinel sites to reflect the potential HAB in harvest waters. Additional water and shellfish sampling are conducted during a HAB. Seafood Branch maintains an Access database of sample results.
    - Chemical Laboratory- This branch performs toxin testing as needed.
    - The Mobile Division Laboratory in the Bureau of Clinical Laboratories identifies and enumerates HAB organisms. Collection materials and sampling instructions are available through the laboratory. Water samples are submitted for a census of dinoflagellates, some diatoms, some flagellates and some cyanobacteria. The lab maintains an Access database of sampling results. Metadata are available.
    - Toxicology Branch- This branch serves as an interface with the public and updates the ADPH web site with Coastal Conditions changes.
    - Baldwin County Health Department environmentalists collect samples from specific BEACH sites as part of surveillance efforts on Alabama's swimming beaches. Environmentalists may respond to complaints by collecting samples of discolored water or floating mats that may be indicative of HAB.
    - Mobile County Health Department environmentalists may respond to complaints by collecting samples of discolored water or floating mats that may be indicative of HAB.
- Alabama Department of Environmental Management

  - ADEM responds to fish kill reports by assessing the site for parameters (temperature, salinity, and dissolved oxygen) that may indicate the source of the kill. Samples for microscopic analysis are collected, and the Department of Conservation and Natural Resources (ADCNR) may be called for consultation. Reports are cataloged in Complaint/Incident Reports quarterly within the agency. Additionally, ADEM voluntarily collects surf water samples at EPA BEACH sites for microscopic phytoplankton exams. Microscopic identification and enumeration are done by ADPH. Samples are collected weekly during the active swimming season and monthly in the winter, giving an indication of phytoplankton from Florida Point at the Alabama state line to the Public Beach on the west end of Dauphin Island.
- Alabama Department of Conservation and Natural Resources
  - The ADCNR investigates fish kills and reports of discolored water and enforces shellfish water closure. ADCNR submits samples to the lab as support for fish kill reports and investigations.
- Dauphin Island Sea Lab
  - Historically, DISL personnel in the MicroAlgal Lab collect samples for HABs under revolving research grants of 2-3 year duration and during HAB event response. Microscopic identification and enumeration was done by ADPH. Collection sites were in the bay waters as well as the off-shore waters. DISL scientists collected water samples for examination as part of research efforts and HAB event response. Extensive water quality data are collected with these samples. DISL toxicologists run brevetoxin tests on shellfish and growing waters as indicators as the bloom progresses.
- The US Food and Drug Administration- Division of Seafood Science and Technology, Chemical Hazards Branch
  - FDA provides toxin testing of shellfish in a *Karenia brevis* HAB event using chemical methods to profile the toxins. Arrangements for analysis are made through the ADPH Seafood Branch to the Chemical Hazards Branch of the Gulf Coast Seafood Laboratory on Dauphin Island.
  - Shellfish specialists provide assistance to the Shellfish Authority to ensure growing areas are managed appropriately during a *Karenia brevis* event.
- National Oceanographic and Atmospheric Administration (NOAA)
  - NOAA prepares a satellite-based bloom forecast tool.
  - The Mobile Lab sends *K. brevis* data with Lat/Long coordinates to assist in the preparation of this bulletin.
  - Sponsor organization for AVMMN
- Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute
  - Toxin analysis and mouse bioassay for shellfish
  - ADPH Seafood Branch makes arrangements with FWRI and collects shellfish samples for analysis.



Coastal Alabama, showing sites routinely monitored for HABs by the agencies involved in the Alabama HAB Management Plan.

Agencies and Personnel

Agency	Contact	Email	Telephone	Address
ADPH Seafood Branch	Byron Webb Ron Dawsey	Byron.webb@adph.state.al.us Ron.dawsey@adph.state.al.us	251 662-7511	
ADPH Chemical Laboratory Cyanotoxins	Mike Huff	mike.huff@adph.state.al.us	334 206-5973	Bureau of Clinical Laboratories 8140 AUM Drive Montgomery
ADPH Toxicology Branch	John Guarisco	John.guarisco@adph.state.al.us	334-206-5022	201 Monroe Street Montgomery, AL 1450R
ADPH Mobile Division Lab	Drew Sheehan	drew.sheehan@adph.state.al.us	251 344-6049	757 Museum Drive Mobile, AL 36608
ADPH Baldwin Co Health Department ADPH Baldwin Co Health Department	Loren Powers	Loren.Powers@adph.state.al.us	251 947-3618	Baldwin Co. Health Dept. Environmental Office PO Box 369 Robertsdale, AL 36567
ADPH Mobile Co. Health Dept.	Kelly Warren	kwarren@mchd.org		
ADEM Mobile Office	Scott Brown Mark Ornellas Susan Rice	JSB@adem.state.al.us MEO@adem.state.al.us srice@adem.state.al.us	251 432-6533 251 450-3400	4171 Commanders Drive Mobile, Alabama 36615-1421 ADEM 2204 Perimeter Rd Mobile AL 36615
ADCNR	Chris	Chris.blankenship@dcnr.alabama.gov		

Marine Resources Dauphin Island	Blankenship Jason Herrmann	Jason.herrmann@dcnr.alabama.gov	251 861-2882 251 861-8741 fax	Dauphin Island
ADCNR Gulf Shores	Chris Denson Kevin Anson	Chris.denson@dcnr.alabama.gov Kevin.anson@dcnr.alabama.gov	251 968-7575 251 968-7307 fax	Gulf Shores
Dauphin Island Sea Lab Marine Ecotoxicology Lab	Alison Robertson	arobertson@disl.org	251-861-2141 x 2142	**Dauphin Island**
FDA /CFSAN/ Division of Seafood Science and Technology/Chemical Hazards Branch			251 690-3368 251 690-3403	1 Iberville Drive Dauphin Island, AL 36528
FDA Shellfish Specialist	David Wiggins	David.wiggins@fda.hhs.gov	850 942-8323 850 942-8326 Fax	USFDA 227 N. Bronough St, Suite 4150 Tallahassee, FL 32301
FL Fish and Wildlife Conservation Commission Fish and Wildlife Institute (Brevetoxin Analysis)	Leanne Flewelling Paula Scott	Leanne.Flewelling@MyFWC.com Paula.Scott@MyFWC.com	727 896-8626 Ext. 1564	Florida Fish and Wildlife Conservation Commission Fish and Wildlife Research Institute 100 8th Ave SE St. Petersburg, FL 33701

Media and Outreach Contacts Including Municipalities and Citizens' Groups

Agency or Organization	Name	Email	Telephone	Address
ADPH Health Promotion and Chronic Disease	Arrol Sheehan	Arrol.sheehan@adph.state.al.us	334 206-5510	The RSA Tower 201 Monroe St Montgomery, AL 36104
Mobile Co. Health Dept.	Stephanie Woods Kelly Warren	swoods@mobilecountyhealth.org kwarren@mchd.org.	251 690-8823	P.O. Box 2867 Mobile AL 36652-2867
City of Gulf Shores		http://www.cityofgulfshores.org/	251 968-2425	P.O. Box 299 Gulf Shores, AL 36547
City of Orange Beach	Phillip West	http://www.cityoforangebeach.com/	251 981-6979	P.O. Box 458 Orange, Beach, AL 36561
Wolf Bay Watershed Watch	Leslie Lassiter Gahagan			
Weeks Bay National Estuary Research Reserve	Scott Phipps	http://www.weeksbay.org/	251-990-5004	Weeks Bay Foundation 11401 US Highway 98 Fairhope, AL 36532
Little Lagoon	Dennis Hatfield	http://www.llps.us/	(251) 942 2233	Little Lagoon Preservation Society, Inc. PO Box 3193 Gulf Shores, Alabama 36547

Known and Potential Harmful Algal Bloom Organisms

Several potentially toxic organisms have been identified in Alabama waters. Toxicity is not always expressed. The principle organism of interest is *Karenia brevis*, which affects shellfish harvest, fish populations, and beach areas. Below are listed some of these toxic organisms identified in estuarine and marine waters.

Organism	Type	Toxin or Effect	Illness or Event
Alexandrium monilatum	Dinoflagellate	Ichthyotoxins	Fish kills
Dinophysis acuminate complex	Dinoflagellate	Okadaic Acid	Diarrhetic Shellfish Poisoning
Dinophysis caudata	Dinoflagellate	Okadaic Acid	Diarrhetic Shellfish Poisoning
Dinophysis ovum	Dinoflagellate	Okadaic Acid	Diarrhetic Shellfish Poisoning
Karenia brevis	Dinoflagellate	Brevetoxins	Neurotoxic Shellfish Poisoning
Karenia spp.	Dinoflagellate	Ichthyotoxins	Fish kills
Karlodinium veneficum	Dinoflagellate	Karlotoxin	Fish kills
Prorocentrum minimum	Dinoflagellate	Venerupin, low dissolved oxygen	"Mahogany Tide" Fish kill
Pyrodinium bahamense	Dinoflagellate	Saxitoxins	Paralytic Shellfish Poisoning
Pseudonitzschia spp	Diatom	Domoic Acid	Amnesiac Shellfish Poisoning
Anabaena	Cyanobacterium	Suite of toxins including hepatotocins, saxitoxin, and dermatotoxins	Neurotoxic and liver effects
Lyngbya	Cyanobacterium	Suite of toxins including dermatotoxins	Swimmer's itch Gastrointestinal inflammation
Oscillatoria	Cyanobacterium	Suite of toxins including anatoxins and hepatotoxins (microcystins)	Neurotoxic and liver effects
Chattonella subsalsa	Flagellate (Rhaphidiphyte)	Brevetoxins, Low Dissolved Oxygen	Fish kills

If an HAB is suspected…

1. The ADPH Mobile Lab supplies collection protocols, collection bottles and preservatives, and standard report forms.

2. Contact the ADPH lab to arrange for a microbiologist to receive and examine the samples. If collection supplies are not on hand, they can be obtained from the lab.

3. If there is a fish kill, identify the fish types. Collect a sample of the dead fish if possible (optional)

4. Document the sampling location by site name and latitude /longitude coordinates if possible.

5. Record the physical data of the area with regard to the extent of the bloom or fish kill, water flow, activities in the area, the water depth (shallow or deep), air temperature. Is there respiratory or eye irritation from aerosols, etc?

6. Record water quality data such as temperature, salinity, color, turbidity, etc.

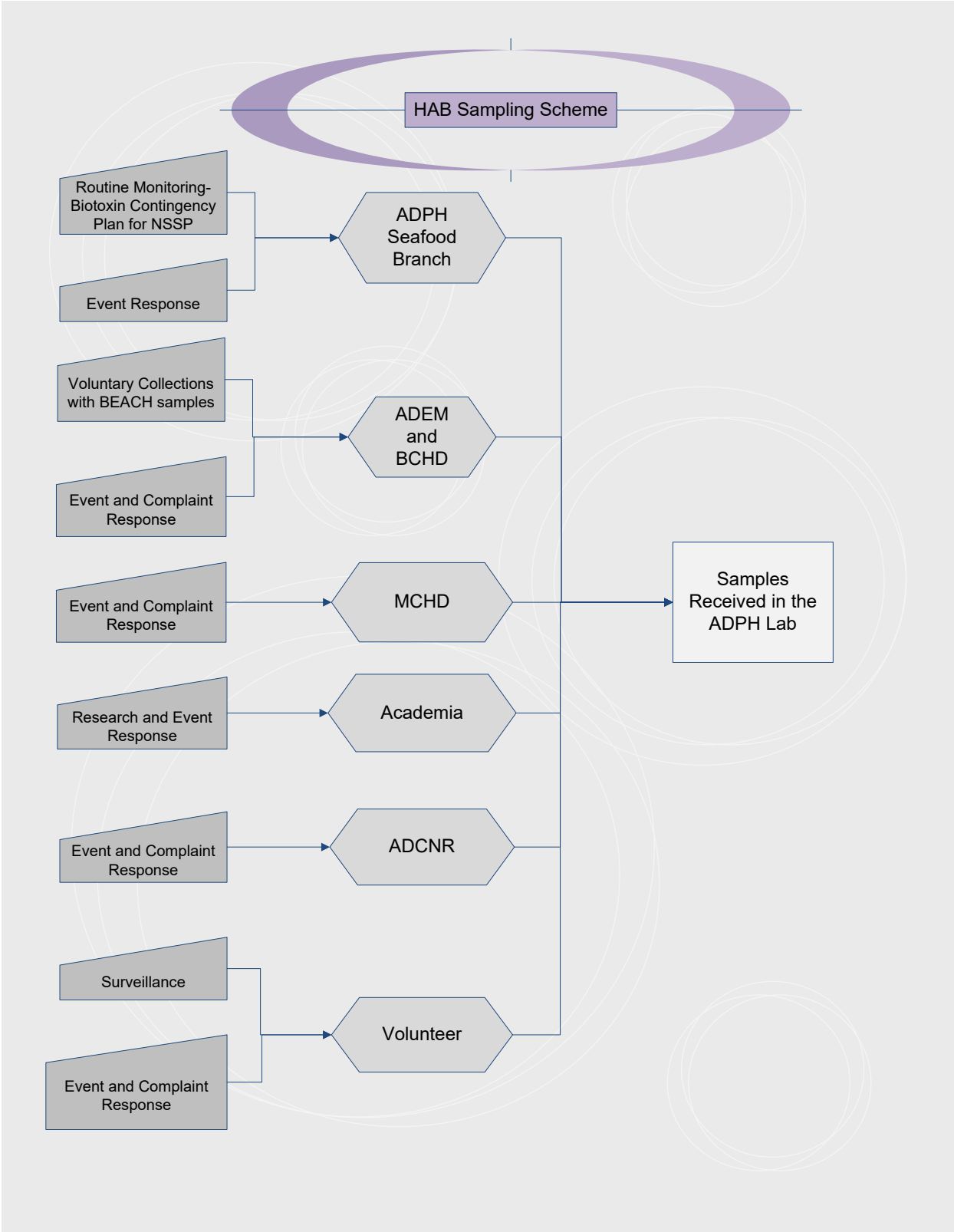
7. Wear gloves to collect samples.

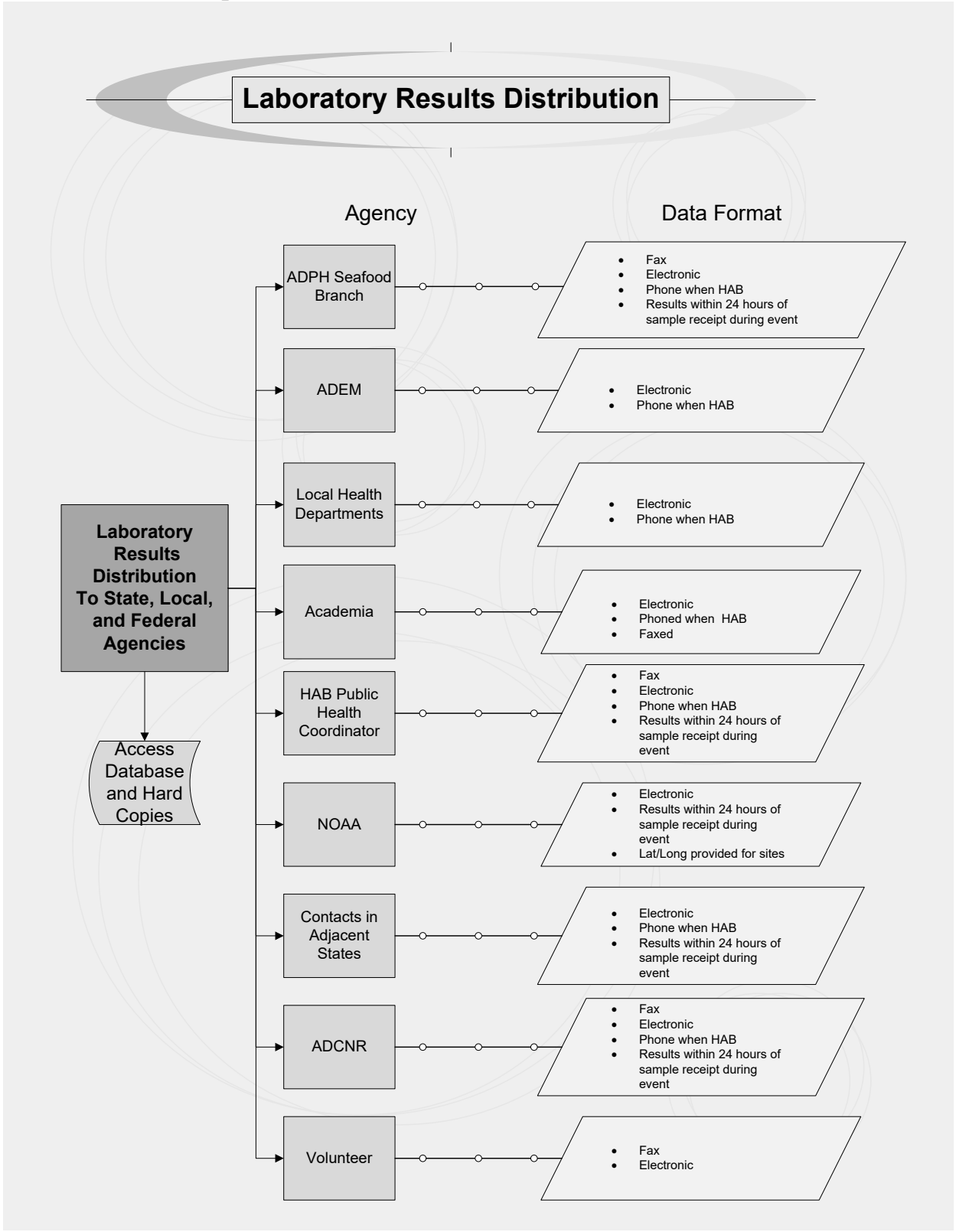
8. Collect samples for microalgal identification, live and preserved. Glass containers are best for live and preserved. Live samples are important for determining color and motility in most phytoplankton and sheath formation in cyanos. Preserved samples are for biomass quantification and multi-species identification.
    a. Plastic drinking water bottles may be used for live samples if lab sample bottles are not available.
    b. Lugol's iodine is the preservative of choice for phytoplankton samples at the ADPH Mobile lab. The preserved sample with adequate Lugol's iodine is the color of strong tea or about 7 mls per liter.

9. Store samples appropriately and transport to the lab as soon as possible
    a. Live samples should be cooled but not iced. Maintaining a temperature similar to the bloom condition is preferred. Wrap bottles in wet newspaper and transport in a dark box at ambient temperature. Counts cannot be reported for live samples.
    b. Preserved samples are protected from light and shipped at ambient temperature.

10. Provide contact information so that results may be called, faxed, or emailed. Note chain of command contacts as necessary.

11. If toxicity studies are necessary, obtain collection bottles and follow protocol provided by the lab.


HAB Sampling Scheme
Routine Monitoring-Biotoxin Contingency Plan for NSSP
Event Response
ADPH Seafood Branch
Voluntary Collections with BEACH samples
Event and Complaint Response
ADEM and BCHD
Event and Complaint Response
MCHD
Research and Event Response
Academia
Event and Complaint Response
ADCNR
Surveillance
Event and Complaint Response
Volunteer
Samples Received in the ADPH Lab


Laboratory Results Distribution
Agency
Data Format
Laboratory Results Distribution To State, Local, and Federal Agencies
Access Database and Hard Copies
ADPH Seafood Branch
Fax
Electronic
Phone when HAB
Results within 24 hours of sample receipt during event
ADEM
Electronic
Phone when HAB
Local Health Departments
Electronic
Phone when HAB
Academia
Electronic
Phoned when HAB
Faxed
HAB Public Health Coordinator
Fax
Electronic
Phone when HAB
Results within 24 hours of sample receipt during event
NOAA
Electronic
Results within 24 hours of sample receipt during event
Lat/Long provided for sites
Contacts in Adjacent States
Electronic
Phone when HAB
Results within 24 hours of sample receipt during event
ADCNR
Fax
Electronic
Phone when HAB
Results within 24 hours of sample receipt during event
Volunteer
Fax
Electronic

HAB Public Health Coordinator

The coordinator duties are:

- To maintain the HAB Contact information so that it will be current and communication lines are open. This will result in regular communication with designated contacts at various agencies.
- To coordinate the sampling activities during an HAB event so that sampling efforts adequately cover the affected areas and duplicate samples are not collected at sites by different agencies. This will help ensure timely analysis, results distribution, and information updates.
- To notify ADPH spokesmen so that agencies, municipalities, civic associations, and the public are given the most current information and possible public health outcomes of an HAB event.
- To maintain a database of sample data, events, notifications, advisories, toxin analyses, and contacts that can serve as a centralized information source for use by government agencies in policy and procedure development and research into modeling, bloom initiation, and outcomes. The coordinator would not be required to keep all data on-site, but maintain current contacts so that the requested data can be located.

Reporting HAB Sample Results and Advisory Triggers

The ADPH Mobile Division Laboratory reports dinoflagellates and diatom cell counts in cells per liter. *Karenia brevis* counts trigger shellfish growing area closures and have resulted in the issuance of public health advisories for swimming areas. The following table was developed by Fish and Wildlife Research Institute of Florida Fish and Wildlife Conservation Commission and used with permission to assist with describing *Karenia brevis* bloom conditions and the possible resulting effects.

Description	*Karenia brevis* (cells/liter)	Possible Effects *(K.brevis* only)
PRESENT	Background levels of 1,000 cells or less	None
VERY LOWa	>1,000 to <5,000	Possible respiratory irritation
VERY LOWb	5,000 to 10,000	Possible respiratory irritation and shellfish harvesting closures
LOWa	>10,000 to <50,000	Respiratory irritation, but chlorophyll levels too low to be detected by satellites
LOWb	50,000 to <100,000	Respiratory irritation, maybe fish kills, and bloom chlorophyll probably detected by satellites
MEDIUM	100,000 to <1,000,000	Respiratory irritation and probable fish kills
HIGH	≥1,000,000	As above plus discoloration

Karenia cell counts in the Very Low or Low a range of 5,000 to 50,000 cells per liter should

1. trigger public health notifications for shellfish growing area closures, beach goers and residents on signs at the beach site
2. Case by case determinations for increased sampling will depend on public complaints, fish kills, and /or discolored water. Samples will be taken at a minimum of weekly until the bloom dissipates
3. Posting of *Karenia* Bloom Description on (ADPH or ADEM) webpage with timely updates.

Karenia counts in the Low b or greater range (exceeding 50,000 cells per liter) at 3 of the 10 Gulf BEACH sites should

1. Trigger a Public Health Advisory notifying the public that respiratory irritation will probably occur and suggestions that reduced exposure to the water and mist will lessen symptoms.
2. initiate a sampling regimen along the Gulf beaches and Perdido Bay of at least once per week until cell counts diminish to Very Low a (>1,000 to <5,000) levels..
3. Post signs at the beach sites indicating the presence of *Karenia* in densities which may cause respiratory irritation.
4. Posting of the description (see above) of *Karenia* on (ADPH or ADEM) webpage with timely updates.

Non-Karenia HAB

1. On-site notification when an HAB such as Pseudonitzschia or cyanobacteria meets or exceeds the World Health Organization (WHO) guideline of 1,000,000 cells per liter or there is obvious scum or rafts of algae floating in the water. Toxin testing may be initiated.

HAB Signage

"Potentially harmful algae have been detected in these waters and contact with the water or aerosols may cause adverse health effects. The Alabama Dept of Public Health continues to monitor the waters and the most current data are available at www.adph.org."

HAB Public Health Advisory Guidance

The Alabama Department of Public Health responds to harmful algal events in two areas, shellfish growing areas and beaches.

Shellfish Growing Areas

The closure of shellfish growing areas in Alabama is codified in the Guide for the Control of Molluscan Shellfish. This procedure provides for closure by the State Health Officer when *Karenia brevis* cell counts per liter exceed 5,000 in the water column. Growing areas are not reopened until brevetoxin levels in molluscan shellfish are less than 20 mouse units and there are less than 5000 cells per liter in the water column. The State Health Officers issue a reopening order for the area.

ALABAMA DEPARTMENT OF PUBLIC HEALTH

The RSA Tower, 201 Monroe Street, P.O. Box 303017, Montgomery, AL 36130-3017

(334) 206-5300 • FAX (334) 206-5534 Web Site: http://www.adph.org

NEWS RELEASE

Shellfish growing waters closed

FOR IMMEDIATE RELEASE
CONTACT: Greg Dunn
ADPH Seafood Branch
(251) 331-0409

The Alabama Department of Public Health has closed shellfish growing waters in Mobile and Baldwin counties. Areas III and IV are closed. This includes Bon Secour Bay. Areas I and II, which include Cedar Point, Heron Bay, Portersville Bay and Grand Bay, remain open at this time.

The order by Acting State Health Officer Dr. Thomas Miller closing harvesting as of 3 p.m. on [November 17, 2015]. The order is issued as a result of the presence of red tide cells *(Karenia brevis)* exceeding standards in Area III.

The Alabama Department of Public Health will continue to monitor bay waters and the shellfish. Harvesting can resume as soon as areas meet acceptable standards.

For additional information concerning the closure, contact Greg Dunn Alabama Department of Public Health, at (251) 662-7511.

11/17/15

HAB Public Health Advisory Guidance
Beach and Swimming Areas

Public notification of HAB and public advisories serve to provide information to bathers and beach residents about the status of HAB at a site and the possible effects that may occur with exposure to the toxins.

ALABAMA DEPARTMENT OF PUBLIC HEALTH

The RSA Tower, 201 Monroe Street, P.O. Box 303017, Montgomery, AL 36130-3017

(334) 206-5300 • FAX (334) 206-5534 Web Site: http://www.adph.org

NEWS RELEASE

Red tide cells remain at the Baldwin County beaches, Alabama Point, Gulf State Park Pavilion, and Cotton Bayou.

FOR IMMEDIATE RELEASE

CONTACT: John Guarisco
ADPH Toxicology Branch
(334)206-5022
Ron Dawsey
ADPH environmental Services
(251) 206-5375

The Alabama Department of Public Health cautions the public that water samples collected this week at Alabama Point, Gulf State Park, and Cotton Bayou in Baldwin County beaches indicate the presence of red tide cells and persons with respiratory problems or those experiencing symptoms of nose, throat or eye irritation should avoid the mist.

Red tide results from a massive build-up of certain species of microscopic sea organisms known as dinoflagellates. These organisms produce a toxin that affects the central nervous system of fish so they are paralyzed and cannot breathe. At high concentrations, the organisms may produce a discoloration of the water. Red tides are often referred to as “blooms.” The species (*Karenia brevis),* isolated from Gulf waters may produce toxins that also cause skin irritation and respiratory problems in humans.

Health Department officials advise:

• Avoid the area if you are susceptible to respiratory problems such as asthma or emphysema.
• Leave the water if you experience skin irritations while swimming or boating and rinse immediately with fresh water.
• If you experience nose, throat or eye irritation when exposed to the gulf mist, avoid the mist.

The Alabama Department of Public Health will continue to monitor gulf and bay waters for the presence of red tide cells.

Unfortunately, the presence of red tide cannot be predicted to be at a certain location at a certain time. The effects depend on many variables such as temperature, salinity, direction of the wind, and how concentrated the organisms are at a given location.
(Date of Issue)

References

1. Model Ordinance 2013
2. Steidinger, K.A. and H.L.Melton Penta Ed., 1999. Harmful Microalgae and Associated Public Health Risks in the Gulf of Mexico
3. Steidinger, K.A., Personal Communication, Taxonomy Training Course, December 2011.
4. Wolny, J. Personal Communication, Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, 2006.
5. Smith, W.L. and C.P. Dorsey. 2015. Phytoplankton Identification Manual. Standard Operating Procedures, Mobile Division Laboratory.
6. Harmful Algal Blooms Observing System (HABSOS), Workshop Report, 2000.
7. Manual on Harmful Marine Microalgae, 2003, UNESCO
8. Aquatic Toxins, Florida webpage, http://www.doh.state.fl.us/Environment/medicine/aquatic/
9. Primer for Harmful Algal Blooms, Gulf of Mexico Alliance/ Gulf of Mexico Coastal Ocean Observing System http://gcoos.tamu.edu/?page_id=2796

Phytoplankton Sampling Protocol

Alabama Dept. of Public Health - Bureau of Clinical Laboratories
Mobile Regional Laboratory
757 Museum Dr. Mobile, AL 36608
Telephone (251) 344-6049 FAX (251) 344-6895 E-mail carol.dorsey@adph.state.al.us

Please make prior arrangements with the lab before bringing in samples to be examined for dinoflagellates. Samples should be transported to the lab as soon as possible.
Sampling Supplies

The laboratory supplies the following items for sample collection:

- Glass sample bottles for phytoplankton monitoring or plastic bottles for toxicity studies
- Small test tube of Lugol's iodine (approx. 7 mls.)
- Sample identification tags
- Laboratory request form

You may also need the following items:

- A box and or cooler for transporting samples to the lab.
- Preserved samples may be transported at ambient temperatures or cooled.
- Live (unpreserved) samples should be cooled (not iced) over ice packs in an insulated container.
- Thermometer for hydrographic data; Salinometer or refractometer
-

Sample Collection

Phytoplankton samples may be collected from the surface during mid-day hours or from multiple depths if necessary or as appropriate for a particular organism. Samples may be collected in the sample bottle (surface) or a special sampler at prescribed depths for transfer to the glass sample bottle. Identify the samples with location (site name, GPS coordinates, etc.) and other pertinent data on the tag and the request form.

- Location, date and time
- Water color, clarity and any odor
- Number, size and species of affected organisms
- Recent weather
- Condition and behavior of animals or organisms
- Are plants or other organisms affected?

Preserved samples for identification and enumeration

. Samples are preserved with Lugol's iodine immediately by pouring the contents of the small test tube directly into the sample bottle then adding sample water to fill to the top of the jar. Use care in handling to avoid contact with the iodine. Replace the cap and invert the bottle gently several times to mix the iodine into the sample.

Live samples for identification only

Fill sample bottles full. If lab bottles are not available, use a clear plastic "bottled water" container. . Wrap bottles with damp newspaper and keep at ambient temperature. Please avoid temperature extremes. Transport to the lab within 24 hours of collection.

Samples for Toxicity Studies

Collect 500 to 1000 mls of water in a plastic bottle (brown is preferred). Allow room for expansion because the samples will be frozen for preservation. Label appropriately.

Salinity

Salinity is helpful in phytoplankton studies. If not measured on-site, you may collect a separate salinity sample (plastic bottle) so that the lab can perform the measurement. Please attach to the dino sample or label with the location.

Karenia brevis Enumeration Procedure

1. Background Information

    1.1. *Karenia brevis* is the dinoflagellate responsible for Neurotoxic Shellfish Poisoning (NSP), respiratory irritation, and animal mortality. 'Red tide' is the common term used to describe this harmful algal bloom or increased concentration of microalgae. During harmful algal blooms cell concentrations may reach millions of cells per liter.
    1.2. "The NSSP Model Ordinance mandates that growing areas be placed in the closed status when cell counts for members of the genus *Karenia* in the water column exceed 5,000 cells per liter of water." (Guidance for the Developing Marine Biotoxin Contingency Plans)
    1.3. In Chapter IV @.04 Marine Biotoxin Control C. Closed status of Growing area (1) (b) (ii) For neurotoxic shellfish poisoning (NSP). The harvesting of shellstock shall not be allowed when: the cell counts for *Karenia brevis* organisms in the water column exceed 5,000 per liter.
    1.4. Microalgae cell counts are performed on preserved samples. A specific aliquot of sample is settled in a chamber so that cells will fall to the bottom and quantification is possible.
    1.5. Sedimentation chambers are manufactured to accommodate specific aliqots of sample.
        1.5.1. Some examples of sedimentation chambers are haemocytometers (0.1 ml), Sedgewick- Rafter (1 ml), Palmer-Maloney and Nalge Nunc tissue culture chambers (3 or 11 mls) and Utermöhl.
        1.5.2. Sedimentation times vary with the amount of sample and the depth and width of the sedimentation chamber. The rule of thumb is 1 hour per inch.
    1.6. Cell counts are adjusted to account for the amount of sample and the dilution of seawater if cell concentrations are expected to be high. Final counts are reported in units of cells per liter.
    1.7. This protocol describes quantifying cells using the Nalge Nunc sedimentation chambers.
2. Equipment
    2.1. Inverted light microscope, preferably with phase contrast illumination, with objectives sufficient to identify distinguishing characteristics of the *Karenia* species.
        2.1.1. 10X, 20X or 40X
        2.1.2. 10X wide field oculars

3. Materials
    3.1. Sample collection jars or bottles with sample identification tags and test request forms
    3.2. Container, such as an ice chest, to store and ship samples to lab in the dark at ambient temperature.
    3.3. Acidified Lugol's iodine sufficient to preserve cells in a water sample

Appendix

1. Model Ordinance Marine Biotoxin Control 2003
2. Laboratory Collection Procedure
3. Laboratory Procedure for the Enumeration of *Karenia brevis*
4. ADPH Mobile Lab HAB Database screen shot
5. Red Tide and Harmful Algal Bloom Frequently Asked Questions- HAB FAQ

II.02 Guidance for Developing Marine Biotoxin Contingency Plans

@.04 Marine Biotoxin Control.

A. Contingency Plan.

(1) The Authority shall develop and adopt a marine biotoxin contingency plan for all marine and estuarine shellfish growing areas.

(2) The plan shall define the administrative procedures and resources necessary to accomplish the following:

(a) Initiate an emergency shellfish sampling and assay program;

(b) Close growing areas and embargo shellfish;

(c) Prevent harvesting of contaminated species;

(d) Provide for product recall;

(e) Disseminate information on the occurrences of toxic algal blooms and/or toxicity in shellfish meats to adjacent states, shellfish industry, and local health agencies; and

(f) Coordinate control actions taken by Authorities and federal agencies.

(3) Except that the Authority shall classify as prohibited any growing areas where shellfish are so highly or frequently affected by marine biotoxins that the situation cannot be safety managed, the presence of marine biotoxins shall not affect the classification of the shellfish growing area under §.03. The Authority may use the conditionally approved classification for areas affected by marine biotoxins.

(4) The plan may include agreements or memoranda of understanding, between the Authority and individual shellfish harvesters, to allow harvesting in designated parts of a growing area while other parts of the growing area are placed in the closed status. Such controlled harvesting shall be conducted with strict assurances of safety, such as by batch release of shellfish lots only after samples of each lot are tested and found to be below the action levels specified in §C.

B. Marine Biotoxin Monitoring. In those areas where marine biotoxins are likely to occur in shellfish, representative samples of shellfish shall be collected during all harvest periods. Samples shall be collected from indicator stations at intervals determined by the Authority, and assayed for the presence of toxins in accordance with §C.

C. Closed Status of Growing Areas.

(1) A growing area, or portion(s) thereof as provided in §A.(4), shall be placed in the closed status for the taking of shellstock when the Authority determines that the level of biotoxin present in shellfish

Phytoplankton Sampling Protocol

Alabama Dept. of Public Health - Bureau of Clinical Laboratories
Mobile Division Laboratory
757 Museum Dr. Mobile, AL 36608
Telephone (251) 344-6049 FAX (251) 344-6895 E-mail carol.dorsey@adph.state.al.us

When possible please make prior arrangements with the lab before bringing in samples to be examined for dinoflagellates. Samples should be transported to the lab as soon as possible.

Sampling Supplies

The laboratory supplies the following items for sample collection:

- Glass sample bottles for phytoplankton monitoring or plastic bottles for toxicity studies
- Small test tube of Lugol's iodine (approx. 5 mls.)
- Sample identification tags
- Laboratory report form

You may also need the following items:

- A box and or cooler for transporting samples to the lab.
- Preserved samples may be transported at ambient temperatures or cooled.
- Live (unpreserved) samples should be cooled (not iced) over ice packs in an insulated container.
- Thermometer for hydrographic data
- Salinometer or refractometer
- Bottom sampler (Lemott) for multiple depth sampling

Sample Collection

Phytoplankton samples may be collected from the surface during mid-day hours or from multiple depths if necessary or as appropriate for a particular organism. Samples may be collected in the sample bottle (surface) or a special sampler at prescribed depths for transfer to the glass sample bottle. Identify the samples with location (site name, GPS coordinates, etc.) and other pertinent data on the tag and the report form.

Preserved samples for identification and enumeration

Please fill bottles to the top allowing room for the addition of Lugol's iodine. Samples are preserved with Lugol's iodine immediately by pouring the contents of the small test tube directly into the sample bottle. Use care in handling to avoid contact with the iodine. Replace the cap and invert the bottle gently several times to mix the iodine into the sample.

Live samples for identification only

Live samples are important for cyanobacteria and flagellate identification. Fill live sample bottles about half to two-thirds full. Cool immediately. Insulate the samples from direct exposure to ice or cold packs using cardboard or newspaper. Try to maintain temperatures similar to bloom conditions. Transport to the lab within 24 hours of collection.

Samples for Toxicity Studies

Collect 500 to 1000 mls of water in a plastic bottle (brown is preferred). Allow room for expansion because the samples will be frozen for preservation. Label appropriately.

Salinity

Salinity is helpful in phytoplankton studies. If not measured on-site, you may collect a separate salinity sample (about 25 mls) so that the lab can perform the measurement. Please attach to the dino sample or label with the location.

Rev. 6/13

1. Background Information

1.1. *Karenia brevis* is a dinoflagellate responsible for Neurotoxic Shellfish Poisoning (NSP) respiratory irritation, and animal mortality. Red tide is a term used to describe the harmful bloom or increased concentration of microalgae. Cell concentrations may reach in the millions of cells per liter in these harmful algal blooms.
1.2. "The NSSP Model Ordinance mandates that growing areas be placed in the closed status when cell counts for members of the genus *Karenia* in the water column exceed 5,000 cells per liter of water." (Guidance for the Developing Marine Biotoxin Contingency Plans)
1.3. In Chapter IV @.04 Marine Biotoxin Control C. Closed status of Growing area (1) (b) (ii) the cell counts for *Karenia brevis* organisms in the water column exceed 5,000 per liter.

2. Equipment
2.1. Light microscope, preferably phase contrast illumination, with objectives sufficient to identify distinguishing characteristics of the *Karenia* species.
2.1.1. 10X, 20X or 40X
2.1.2. 10X wide field oculars

3. Materials
3.1. Sample collection jars or bottles with sample identification tags and test request forms
3.2. Container, such as an ice chest, to store and ship samples to lab in the dark.
3.3. Acidified Lugol's iodine sufficient to preserve cells in a water sample
3.3.1. Samples should be preserved immediately upon collection.
3.3.2. The staining of the water should resemble strong tea. Ex:5 mls of Lugol's in 1 liter of seawater.
3.4. Borosilicate tubes with nonreactive liner caps for storing Lugol's iodine.
3.5. Chamber slide to contain 3 or 11 mls of water. Ex: Lab-Tek II by Nalge Nunc
3.6. Cover slips, 24X64 mm
3.7. Pipettes for filling the chamber slides
3.8. Timer for timing the sample settling period
3.9. Counting tally
3.10. Ocular micrometer (recommended)
3.11. Ocular reticule grid
3.12. Dinoflagellate reference materials such as manuals, on-line information, etc.

4. Formulations
Care should be taken to avoid contact with the ingredients of this Lugol's iodine. Wear gloves and appropriate eye protection when handling. Follow standard lab safety practices.

4.1. Acidified Lugol's Iodine
4.1.1. 100 gms Potassium Iodide (KI)
4.1.2. 1 liter deionized water
4.1.3. 50 gms iodine (crystalline)
4.1.4. 100 ml glacial acetic acid
4.1.5. Dissolve KI in deionized water then dissolve iodine crystals. Add glacial acetic acid. As the solution nears saturation, decant so that any possible precipitate is

removed. Store in amber glass at room temperature. Expires two years from preparation.

4.1.6. Dispense sample preservation volumes to nonreactive tubes (glass) and use caps with nonreactive liners. Ex: 5 mls for 1 liter of seawater.

5. Sample Collection
   5.1. Samples should be taken from collection sites representative of the shellfish growing area.
   5.2. Surface grab samples are satisfactory. Fill bottles to the top with seawater.
   5.3. Preserve samples with Lugol's immediately after collection.
   5.4. Store out of the sunlight for transportation back to the lab.
   5.5. Record hydrographic data of the area such as temperature and salinity on an accompanying request form.

6. Chamber set-up
   6.1. The identification of a sample is checked for agreement with the request form when samples are delivered to the lab.
   6.2. The preserved sample is gently mixed by inversion about 10 times to distribute cells evenly throughout the bottle.
   6.3. Prepare a chamber slide by removing the plastic cover and placing a glass cover slip diagonally across the chamber. When the chamber is filled with sample, the cover slip will slide into place on the top of the chamber due to surface tension.
   6.4. Use a pipette of the appropriate dispensing volume fill the chamber.
   6.5. Set a timer for 15-20 minutes to allow the contents of the sample to settle to the bottom.

7. Calculations
   7.1. Chamber factors
      7.1.1. Counting chambers should be checked for the number of reticle grids across and down the chamber for each microscope and analyst. This will be used in cases of high counts when representative fields are counted rather than a whole chamber Ex:
      7.1.2. Calibration of the 11 ml Lab-Tek chamber cell by analyst A gave an area of 800 grids (10 down and 80 across) using the 10X objective and 10X ocular
      7.1.3. 10 grids with an average of 60 cells each are counted
      7.1.4. Multiply 60 cells X 800 to calculate the chamber count = 48,000 Karenia brevis cells per chamber.

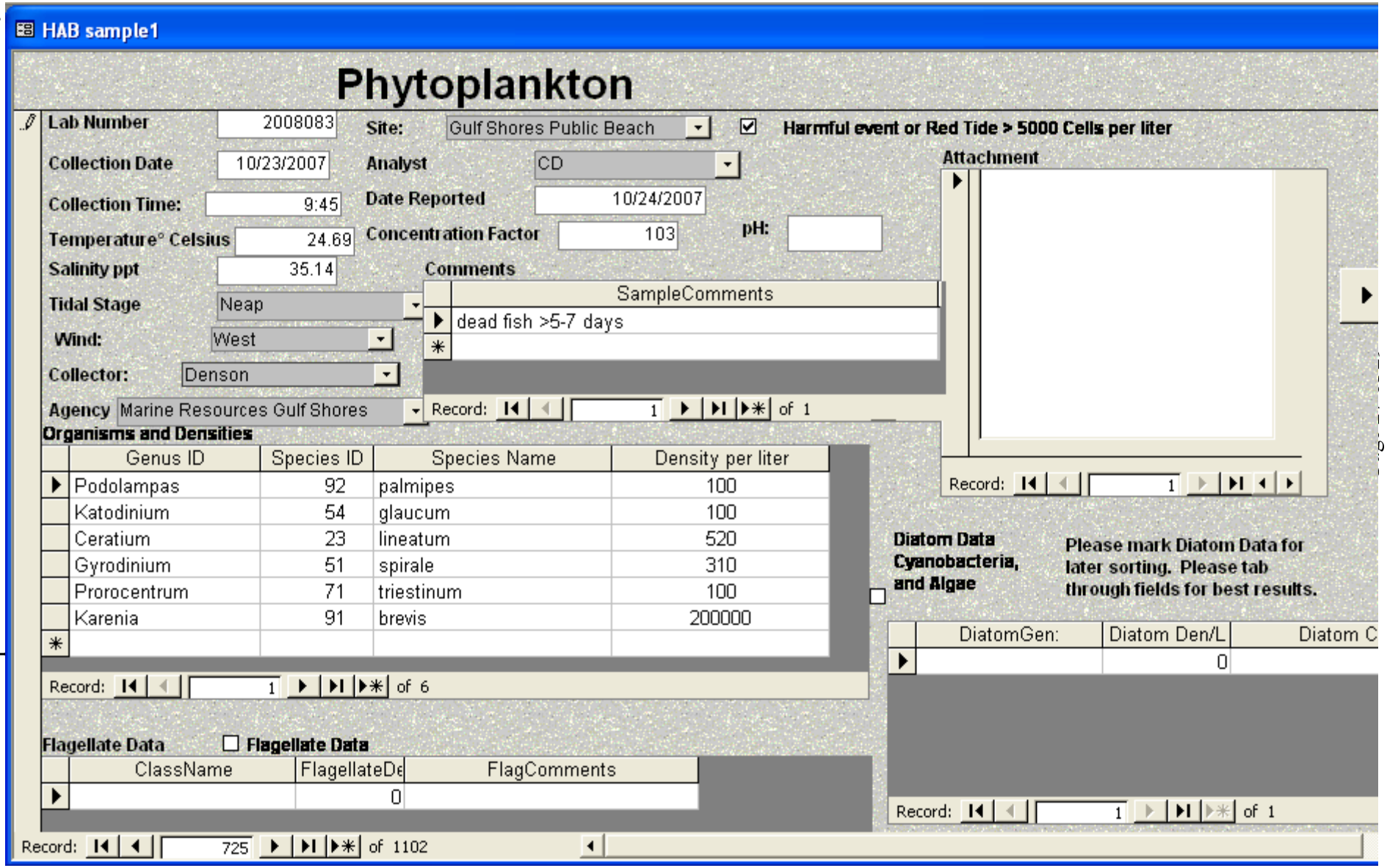
   7.2. Concentration Factor
      7.2.1. The Concentration Factor will correct for the volume of sample counted as a portion of a 1000 ml sample of seawater.
      7.2.2. $\frac{\text{1000 mls}}{\text{mls of seawater counted}}$ = Concentration Factor

8. Counting cells
   8.1. View chambers with the 10 X objective and phase contrast illumination.
   8.2. *Karenia brevis* cells are typically 18-45 μm and stained golden by the Lugol's. Lipid droplets in cytoplasm produce a characteristic refractive or "glowing", and a lacy quality to the cell.
      8.2.1. Other *Karenia* species have distinctive properties. Use reference materials to aid in the identification of all Karenia species.

8.3. Blooms may have multiple *Karenia* species. Enumerate each separately.
8.4. For samples with high cell counts use a reticle grid to count a representative number of fields.
8.4.1. Average the number of cells seen per grid
8.4.2. Apply the Chamber factor to calculate the Raw Cell Count
8.5. Record cell counts
8.6. Multiply by the concentration factor to calculate a raw cell count per liter
8.7. Round counts to two significant figures to avoid overstating the precision of the count. This is the reportable Cell Density per Liter.
8.8. If a sample yields between 3,000 and 7,000 cells per liter, count 3 representative chambers and average for the collection site Cell Density per Liter.

9. Reporting Results
9.1. Report cell densities per liter to the Shellfish Authority

10. References
10.1. Model Ordinance 2003
10.2. Steidinger, K.A. and H.L.Melton Penta Ed., 1999. Harmful Microalgae and Associated Public Health Risks in the Gulf of Mexico
10.3. Wolny, Jennifer .Personal Communication, Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, 2006.
10.4. Smith, William L. and C.P. Dorsey. 2005. Phytoplankton Identification Manual. Standard Operating Procedures, Mobile Division Laboratory.


HAB sample1
Phytoplankton
Lab Number 2008083
Site: Gulf Shores Public Beach
Harmful event or Red Tide > 5000 Cells per liter
Collection Date 10/23/2007
Analyst CD
Collection Time: 9:45
Date Reported 10/24/2007
Temperature° Celsius 24.69
Concentration Factor 103
pH:
Salinity ppt 35.14
Tidal Stage Neap
Wind: West
Collector: Denson
Agency Marine Resources Gulf Shores
Comments
SampleComments
dead fish >5-7 days
Record: 1 of 1
Attachment
Record: 1
Organisms and Densities
Genus ID | Species ID | Species Name | Density per liter
Podolampas | 92 | palmipes | 100
Katodinium | 54 | glaucum | 100
Ceratium | 23 | lineatum | 520
Gyrodinium | 51 | spirale | 310
Prorocentrum | 71 | triestinum | 100
Karenia | 91 | brevis | 200000
Record: 1 of 6
Diatom Data Cyanobacteria, and Algae
Please mark Diatom Data for later sorting. Please tab through fields for best results.
DiatomGen: | Diatom Den/L | Diatom C
0
Record: 1 of 1
Flagellate Data
Flagellate Data
ClassName | FlagellateDe | FlagComments
0
Record: 725 of 1102

Red Tide and Harmful Algal Bloom Frequently Asked Questions – HAB FAQ

1. What are Harmful Algal Blooms (HAB)?

   Harmful algal blooms can be caused by microscopic algae found naturally in Alabama waters. Blooms occur when cells begin to multiply rapidly. This growth explosion produces a mass of organisms which create conditions not usually encountered when few cells are present. The high concentrations of the tiny organisms can result in fish kills from oxygen depletion, or toxin production which may affect animals and humans. Some HABs in our area result in water discoloration and a temporary reduction in fish being caught, because fish leave the area while a bloom is present. Cyanobacteria or blue-green algae may also cause discolored water, scum on the top or floating clumps in the water. Cyanobacteria may also produce toxins which affect humans and animals.

2. What is Florida Red Tide?

   In Alabama waters, Florida Red Tide refers to a particular HAB caused by *Karenia brevis*. This organism is of public health concern because it can produce a toxin that affects the nervous system of fish, can cause skin and respiratory irritation in humans, and accumulate in oysters leading to Neurotoxic Shellfish Poisoning if the toxic shellfish are consumed.

3. What causes HAB and Red Tide?

   The reason for the microscopic algae's explosive growth is not currently known. Researchers are examining many factors such as nutrients in the water, light, temperature, and water mixing, to try to answer this question. The Red Tide does not appear to be caused by environmental changes brought on by people. Red tides have occurred for a long time, and have been recorded in Florida since the 1840's.

4. Can Red Tide or HAB be predicted?

   Red Tide and HAB formation cannot be predicted at present. There is a lot of research being conducted to understand the beginnings of blooms. There are scientific tools that can help us know the general direction of Red Tide movement and, in some cases, how intense the bloom might be along a coastal area. However, these tools cannot predict when the bloom will be at a specific beach, how intense it will be, or how long it will last.

5. What effects do Red Tide and HAB have on humans?

   Karenia brevis, the Red Tide organism, produces a toxin that can cause respiratory effects, such as wheezing and coughing, eye irritation, and skin irritation. This occurs in susceptible people when the microscopic cells number more than 50,000 in a liter of water, and can affect some individuals when cell counts are less. Exposure comes from breathing the aerosols produced when cells are broken up in surf and dispersed by wind. When the red tide toxin is consumed and concentrated by shellfish, such as oysters and clams, eating the shellfish can result in Neurotoxic Shellfish Poisoning. Shellfish harvest is prohibited when a Red Tide is ongoing.

Other HAB effects depend on the type of microscopic algae creating the bloom. Toxins produced by other microalgae can be consumed and concentrated by some shellfish, causing Paralytic Shellfish Poisoning, Diarrheic Shellfish Poisoning, and Amnesic Shellfish Poisoning. You should never eat shellfish harvested from unapproved or closed waters.

6. What should I do if I experience respiratory or skin irritation from Florida Red Tide?
   Leave the area if you are experiencing respiratory or skin irritation. Get out of the water and wash thoroughly. The symptoms should disappear within a couple of hours. If you have chronic respiratory conditions such as asthma or emphysema, you should avoid red tide areas.

7. What is being done to protect the public from the effects of HABs and Red Tide?
   The Alabama Department of Public Health works with other state agencies and academic institutions to monitor the area waters for HAB and Red Tide organisms. When a Red Tide or HAB is detected in shellfish growing areas, the area is closed until the HAB has dispersed, and no toxin is detected in the shellfish. When microscopic cell counts along the coast, the bays, or estuaries are high enough to cause human health effects, the Health Department may post notifications. A Public Health advisory may be sent to the local media for distribution when larger areas of the coast are affected.

8. Shellfish are affected by HAB and Red Tide but what about fin fish?
   Fish do not accumulate the Red Tide toxin like oysters and clams do, so they are safe to eat if they do not appear sick. Never eat fish that are found dead or acting sick when you catch them.

9. Can I eat fish, shrimp, oysters and clams in a restaurant if a Red Tide or HAB are occurring on the beach?
   Yes. Shellfish and fin fish served in restaurants are harvested or caught in areas without Red Tide or HAB contamination.

10. Where can I get information about HAB or Red Tide that might be in Alabama waters?
    The ADPH or ADEM websites

11. Where can I get more information about the effects of toxic HAB events?

    Here are several websites that give information on Red Tide and other microalgal toxins.
    The Centers for Disease Control http://www.cdc.gov/nceh/hsb/hab/default.htm
    Florida Fish and Wildlife Institute
    http://research.myfwc.com/features/view_article.asp?id=9670
    Florida Department of Health Aquatic Toxins-
    http://www.floridahealth.gov/environmental-health/aquatic-toxins/index.html
    Florida's University of Miami Red Tide Poison Control
    1-800-222-1222
    http://www.med.miami.edu/poisoncontrol/x59.xml